Blue No. 1. The fact the K values are smaller than 1.0 for both drugs supports a selective adsorption mechanism rather than complete coverage of the crystal surfaces by the dye.

It has been reported (10–12) that bile salts increase the solubility of poorly soluble drugs by micellar solubilization. Consequently, the dissolution rate is expected to increase in the presence of sodium cholate. The influence of Blue No. 1 on the solubilizing effect by 0.04 M sodium cholate was studied using sulfathiazole Form I (single crystal) as the model substance. Data obtained in Fig. 8 show that Blue No. 1 modified markedly the dissolution kinetics of sulfathiazole in sodium cholate solution. A concentration of 5 mcg./ml. brought the dissolution rate to a value very close to that in distilled water. The solubility of sulfathiazole at 25° was found to be 0.85 mg./ml. in both 0.04 M sodium cholate and in 0.04 M sodium cholate containing 5 mcg./ml. Blue No. 1. In distilled water, the solubility was 0.47 mg./ml. Therefore, the dissolution of sulfathiazole in the cholate–dye system does not follow the laws predicted by simple diffusion kinetics and suggests an interfacial mechanism.

This observation is extremely interesting in relation to the intestinal absorption process in the presence of water-soluble dyes. More studies are necessary for complete characterization of this problem from the physical-chemical standpoint. Such studies will be generally important to the eventual understanding of the role of water-soluble dyes in dissolution and drug transport.

REFERENCES

(1) J. Piccolo and R. Tawashi, *J. Pharm. Sci.*, **59**, 56(1970).

(2) R. Tawashi and J. Piccolo, to be published.

(3) W. I. Higuchi and H. Y. Saad, *J. Pharm. Sci.*, **54**, 74(1965).

(4) H. Y. Saad and W. I. Higuchi, *ibid.*, **54**, 1303(1965).

(5) I. C. Edmundson and K. A. Lees, *J. Pharm. Pharmacol.*, **17**, 193(1964).

(6) M. B. Ives and J. T. Plewes, *J. Chem. Phys.*, **42**, 293(1965).

(7) J. J. Gilman, W. G. Johnston, and G. W. Sears, *J. Appl. Phys.*, **29**, 747(1968).

(8) M. B. Ives, *J. Phys. Chem. Solids*, **24**, 275(1963).

(9) A. S. Michaels and A. R. Colville, Jr., *J. Phys. Chem.*, **64**, 13(1960).

(10) T. R. Bates, M. Gibaldi, and J. F. Kanig, *J. Pharm. Sci.*, **55**, 191(1966).

(11) *Ibid.*, **55**, 901(1966).

(12) T. R. Bates, M. Gibaldi, and J. F. Kanig, *Nature*, **210**, 1331 (1966).

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Survey of a Laboratory Building for Airborne Antibiotics

MARY ANN GARTH, HENRY BRYANT, JULIAN KRAMER, and AMIEL KIRSHBAUM

Abstract □ Antibiotics are routinely examined by laboratory analysis in a six-story building which houses the laboratories of the National Center for Antibiotics and Insulin Analysis (NCAIA) on the second floor. A survey was performed to determine if antibiotic dust was being disseminated throughout the building. Air throughout the building was analyzed both quantitatively and qualitatively for residues of penicillin and tetracyclines. The microbiological assay plates used were sensitive enough to detect 0.001 unit of penicllin and 0.0004 mcg. of chlortetracycline in the air. Results of this study showed that, outside of the actual laboratories of NCAIA, the incidence of antibiotic contamination of the air was negligible. In the immediate areas where antibiotics were physically handled, 0.012 unit of penicillin and 0.0185 mcg. of chlortetracycline per cubic foot of air were detected.

Keyphrases □ Antibiotic, airborne dissemination—laboratory building □ Cultures, plate—antibiotic detection □ Air sampling—vacuum collection through membrane □ Laboratory contamination—antibiotics, airborne

As the use of chemicals becomes more diversified in modern life, the extent to which these substances contaminate the environment becomes a matter of increasing concern. Similarly, for some years, effort has been directed toward the study of the incidence of microbial contamination of the environment, especially in hospitals (1, 2), and the control of this contamination. Recently, attention has been directed toward microbe-free environments for the assembly of aerospace equipment (3, 4).

Work in the laboratories of the National Center for Antibiotics and Insulin Analysis (NCAIA) involves the possibility of a special kind of contamination. Approximately 100 chemists and microbiologists perform laboratory analyses of antibiotic drugs. There is little information concerning the extent to which these antibiotics may permeate the atmosphere as the result of manipulations in the course of analysis. Welch *et al.* (5) demonstrated that a group of antibiotics analysts carried nasal staphylococci possessing a significantly higher incidence of antibiotic resistance than did control groups. The antibiotic resistance was attributed to antibiotic dusts and aerosols, but the amount of antibiotics in the air of the laboratory was not determined. The authors, therefore, surveyed the atmosphere of laboratories and of the entire building in which they are housed for the presence of two of the most frequently tested groups of antibiotics: penicillins and tetracyclines.

NCAIA is located mainly on the second floor of the U. S. Food and Drug Administration (FDA) Building in Washington, D. C.; at the time of this study, it also had ancillary laboratory space on the first and third floors, used for penicillin contamination and for turbidimetric assays, respectively. This modern well-equipped laboratory building consists of six floors, a basement, and a subbasement. All air enters by way of five separate air-conditioning systems using 15 induction fans

Figure 1—*Vacuum system used in the air sampling techniques.*

and leaves with the aid of 56 exhaust fans. There is no recirculation of air. Roll-type filters are used for filtering incoming air. Each room is equipped with induction and exhaust systems and is closed off from adjoining rooms by swinging doors. The total average airflow for the entire building is 683,000 cu. ft./min. for the air conditioning and 700,000 cu. ft./min. for the exhaust system. The average relative humidity is approximately 47% throughout the building.

The study was divided into three phases. The first was a passive fallout test intended to determine the incidence, if any, of antibiotic dust in representative areas of the building. The second was a vacuum filtration of air in areas in which positive results were found in the first phase, other than NCAIA laboratories. In the third phase, every room of NCAIA was monitored for antibiotic fallout, and then quantitative determinations were made of antibiotics per volume of air in those rooms found positive.

MATERIALS AND METHODS

Test Organisms—Since the penicillins and tetracyclines constitute the largest proportion of drugs tested and were therefore the largest source of possible contamination, the test organisms selected were those most sensitive to these antibiotics, namely, *Sarcina lutea* (ATCC 9341) and *Bacillus cereus* var. *mycoides* (ATCC 11778). Zones of inhibition are produced when as little as 0.001 unit of penicillin or 0.0004 mcg. of chlortetracycline (CTC) comes in contact with an agar surface inoculated with test organisms. CTC was selected as a representative of the tetracycline group of antibiotics.

Identification of Penicillin—Penicillinase, an enzyme, will reduce or completely inactivate the antibacterial activity of penicillin. This enzyme was incorporated in some of the test systems to determine if any antibacterial activity observed was, indeed, penicillin.

Tests Plates—*Fallout Plates*—Plates were prepared by adding 8 ml. of agar inoculated with one test organism into each of 90 plastic Petri dishes, 100 × 15 mm. Some plates of each type were modified by the addition of 0.5 ml. of penicillinase to the melted inoculated agar.

Penicillin Plates (*P Plates*): Yeast-Beef Agar (Medium 4), prepared according to CFR Title 21, Section 141.103, was inoculated with 0.1% of an *S. lutea* suspension, prepared according to CFR Title 21, Section 141.104.

CTC Plates (*T Plates*): Base Agar, pH 5.9 (Medium 8), prepared according to CFR Title 21, Section 141.103, was inoculated with 0.1% of a *B. cereus* spore suspension prepared according to CFR Title 21, Section 141.104.

Fallout Plates for Sterile Areas—To avoid microbial contamination of sterile areas, uninoculated fallout plates were used for these locations. Six milliliters of the appropriate uninoculated agar was placed in Petri dishes. These plates were exposed in the respective sterile areas and then covered and returned to the assay laboratory. There the base agar was overlaid with 4 ml. of inoculated agar.

Cylinder Plates—Cylinder plate assays were performed as described in CFR Title 21, Part 141, Subpart B, using the following buffers: (*a*) for penicillin assay, 1% pH 6.0 potassium phosphate buffer (CFR Title 21, Section 141.102, Solution 1); and (*b*) for CTC assay, 0.1 *M* pH 4.5 potassium phosphate buffer (CFR Title 21, Section 141.102, Solution 4).

Air Sampling Equipment—A vacuum system (Fig. 1) was used with a membrane filter with a porosity of 0.22 μ. The filter was placed in a clean support system connected to a vacuum pump through an airflow meter.

Controls—*Negative Control of Fallout Plates*—Each time plates were exposed, a plate prepared in the same way as the exposed plate but left covered was used as a negative control.

Positive Control of Fallout Plates—Each day that plates were exposed, tests were performed to determine their sensitivity to penicillin and tetracycline, as follows:

1. Sodium penicillin G working standard, prepared according to CFR Title 21, Section 141.110(b), was diluted in water to make concentrations of 0.2, 0.1, and 0.05 unit/ml.
2. Chlortetracycline hydrochloride working standard, prepared according to CFR Title 21, Section 141.110(b), was diluted in water to make concentrations of 0.08, 0.04, and 0.02 mcg./ml.

Next, 0.01-ml. portions of each of the penicillin standard solutions were placed directly on the surfaces of duplicate *S. lutea* plates. Similarly, CTC standard solutions were placed on the surface of two *B. cereus* plates. The amounts used were thus 0.002, 0.001, and 0.0005 unit of penicillin and 0.0008, 0.0004, and 0.0002 mcg. of CTC.

Negative Filtration Control—As a negative control, an area was selected that was negative for activity in Phase I. The filtration system was used exactly as for the other sites.

Positive Filtration Control—An area where antibiotic drugs were actively handled was used as the positive control.

Recovery Controls—Membrane filters were spotted with solutions containing varying known amounts of penicillin or CTC. These filters were extracted and the extracts assayed.

EXPERIMENTAL

Phase I—Passive Fallout Phase—The presence of antibiotics in the atmosphere of the building was first determined by exposing the surface of a number of agar plates to fallout from the air. Plates were exposed for 1 hr. in at least five different locations on each of seven floors of the building excluding the subbasement. One plate of each type was exposed at each site. On each of 4 days, one set of plates was exposed in the late morning after work had commenced and another set was exposed in the late afternoon. Figure 2 shows the typical location of plates on one floor. In some rooms where antibiotics were handled, additional plates containing agar with penicillinase added were exposed. After the exposure, all plates were incubated overnight at 30°.

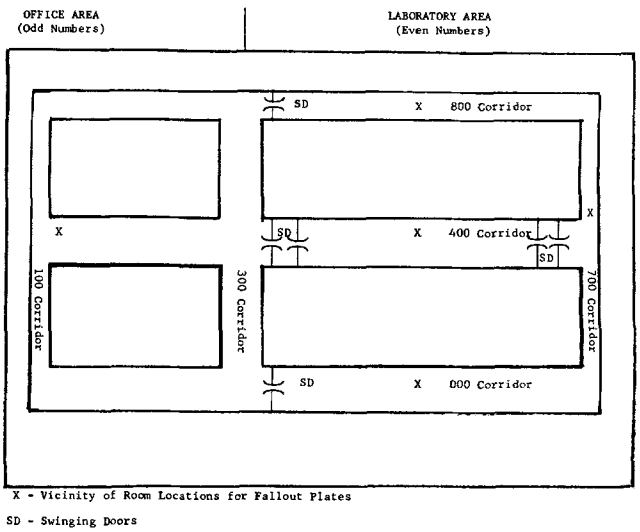

Figure 2—*Location of fallout plates.*

Table I—Antibiotic Fallout: Number of Zones of Inhibition per Plate (Phase I)[a]

Location	Oct. 26 A.M. P	Oct. 26 A.M. T	Oct. 26 P.M. P	Oct. 26 P.M. T	Oct. 31 A.M. P	Oct. 31 A.M. T	Oct. 31 P.M. P	Oct. 31 P.M. T	Nov. 7 A.M. P	Nov. 7 A.M. T	Nov. 7 P.M. P	Nov. 7 P.M. T	Nov. 14 A.M. P	Nov. 14 A.M. T	Nov. 14 P.M. P	Nov. 14 P.M. T	Total
Basement																	
B305	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
B433A	1	0	0	0	1	0	—	—	5	0	3	0	2	0	2	0	14
B042), B672), B756)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
First floor																	
1056	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
1758	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
1464), 1820), 1823)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Second floor																	
2018	N	2	3	3	0	2	N	0	6	0	4	2	1	1	0	0	24 + 2N
2034	—	—	—	—	N	2	N	10	9	3	N	4	4	4	N	4	38 + 4N
2034[b]	—	—	—	—	4	N	0	0	—	—	2	0	0	6	1	5	18 + 1N
2090	0	2	0	0	1	2	3	0	0	0	0	0	0	0	3	6	17
2159	0	0	0	0	0	0	0	0	0	0	0	0	3	0	4	0	7
2417	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2
2426	0	0	1	1	0	0	1	1	0	0	0	1	0	0	0	0	5
2756	—	—	—	—	0	14	0	2	1	0	0	1	0	9	0	4	31
2756[b]	—	—	—	—	—	—	—	—	—	—	—	—	0	4	0	3	7
2804	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	2
2884	1	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	7
Third floor																	
3470	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2
3052), 3321), 3736), 3832)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fourth floor																	
4434	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
4762	0	0	0	0	0	0	0	0	—	—	0	0	1	0	0	0	1
4048), 4415), 4834)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fifth floor																	
5417	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	2
5046), 5758), 5807), 5852)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sixth floor																	
6415	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
6426	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	3
6840	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	2
6018), 6747)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] P = *S. lutea* plates (for detecting penicillin), T = *B. cereus* plates (for detecting antibiotics of the tetracycline group), N = numerous zones, unable to count, and — = not done. [b] Penicillinase plates.

Phase II—Vacuum Sampling of Air at Sites Outside of NCAIA—The locations selected for this part of the study were those which had shown positive results in the first phase, other than NCAIA laboratories. Measured volumes of air were sampled. Air was allowed to flow through the filter for approximately 1 hr. Flow meter and vacuum pump meter readings were recorded at the beginning and end of each sampling period. The length of the sampling period was noted and used with the airflow data to calculate the total volume of air filtered.

Two filters were exposed at each test site, one immediately fol-

Table II—Antibiotics Found by Air Filtration (Phase II)

Date	Room	Penicillin cu. ft. Tested	Penicillin Number of Zones	Tetracyclines cu. ft. Tested	Tetracyclines Number of Zones
11/29	B433A	34	Numerous	35	1
11/29	B305	34	Negative	33	Negative
12/7	1056	33	Negative	32	Negative
12/8	1758	32	Negative	29	Negative
12/8	3470	33	Negative	32	Negative
12/12	4762	33	Negative	32	Negative
12/14	4434	33	Negative	33	Negative
12/14	5417	32	Negative	33	Negative
12/18	6426	31	Negative	30	Negative
12/18	6415	30	Negative	30	Negative
12/19	6840	32	Negative	31	Negative
11/22	2018 (positive control)	32	Numerous	32	Negative
11/24	4415 (negative control)	35	Negative	32	Negative

Table III—Number of Antibacterial Spots Found in Fallout Plates in NCAIA Area (Phase III)[a]

Room	Day 1 P	Day 1 T	Day 2 P	Day 2 T	Room	Day 1 P	Day 1 T	Day 2 P	Day 2 T
1016	0	0	2	0	2468	1	0	1	0
2010	N	N	N	N	2472	2	0	0	0
2012	N	N	N	N	2474	0	0	0	0
2014	N	3	10	4	2476	7	1	0	0
2016	1	2	4	0	2720	3	3	4	N
2018	2	0	2	0	2732	0	0	0	0
2024	6	4	4	2	2740	2	1	N	0
2026	N	0	N	0	2756	0	2	0	1
2032	12	7	3	2	2766	3	1	0	0
2034	9	10	N	12	2768	0	0	1	0
2042	5	6	N	11	2772	0	0	0	0
2046	0	0	0	0	2803	0	0	0	0
2058	0	1	0	0	2804	1	1	4	4
2062	1	0	4	0	2813	0	0	4	1
2066	1	0	N	0	2819	0	0	1	0
2074	8	1	6	0	2821	2	0	0	0
2082	0	2	1	0	2825	0	0	0	0
2086	1	2	1	2	2826	0	0	0	1
2090	0	0	0	1	2827	0	0	0	0
2159	0	0	0	0	2831	0	1	2	0
2161	0	0	0	0	2846	0	1	0	0
2169	0	0	0	0	2850	2	17	0	0
2410	1	0	0	0	2858	1	0	3	0
2414	1	3	1	0	2874	0	0	0	0
2415	N	8	N	5	2884	0	0	0	0
2420	0	0	0	0	3054	0	9	0	0
2422	10	1	2	0	3058	0	0	0	0
2426	0	0	0	0	3062	0	0	1	1
2427	0	0	0	0	3068	0	0	8	0
2432	0	0	0	0	3070	0	1	3	2
2434	0	0	0	0	3074	0	0	0	0
2438	1	0	0	0	Totals	84	88	74	49
2448	1	0	2	0		+5N	+2N	+8N	+3N

[a] P = *S. lutea* plates, T = *B. cereus* plates, and N = numerous, unable to count.

lowing the other. The setup was changed after each exposure. The filter was removed for testing and the rest of the equipment was thoroughly washed before reuse. Each filter was placed directly on the agar surface of the appropriate test plate. One filter from each location was placed on a P plate; the other from the same location was placed on a T plate. The plates were then incubated overnight at 30°. Negative and positive filtration controls were also processed.

Phase III—Measurements in Every NCAIA Room—Phase III was divided into two tests. First, fallout plates, prepared as in Phase I, were placed in every NCAIA room and laboratory. Then a quantitative air-filtration study was undertaken as a followup to the fallout results in areas where positive results were most consistently obtained.

Two air filtrations were done at each test site, one to determine penicillin and the other to determine tetracycline activity. After filtration the membrane filter was removed from the holder and extracted with 4.0 ml. of the appropriate buffer by shaking with a wrist-action shaker for 30 min. If the resultant solution was to be assayed for tetracycline activity, penicillinase was added to inactivate any penicillin that might have been picked up in the filtration. If the solution was to be tested for penicillin, the solution was divided into two aliquots; 0.5 ml. of penicillinase was added to one aliquot and the other was left untreated. Both aliquots were then tested. A positive test result for the untreated aliquot and a negative result for the aliquot treated with penicillinase identified the positive result as penicillin.

Table IV—Amounts of Antibiotics Found in Areas with Most Spots of Fallout Plates in NCAIA (Phase III)

Date	Room	Penicillin cu. ft.	Penicillin unit/cu. ft.	Tetracyclines cu. ft.	Tetracyclines mcg./cu. ft.
1/29	2010	35	0.009	32	0.0185
1/30	2012	31	0.003	32	0.003
1/30	2014	33	0.012	33	0.002
2/6	2026	32	0.004	33	0
2/6	2034	33	0.012	32	0
2/8	2042	32	0.009	32	0
2/8	2415	34	0	32	0
2/9	2720	32	0.0025	32	0
2/9	2740	33	0	32	0

RESULTS

In Phase I, as shown in Table I, numerous zones of inhibition were found in plates exposed in laboratories where antibiotics were regularly tested (second floor).

To determine qualitatively the incidence of airborne penicillin in a room where large amounts of antibiotics were handled, some plates were exposed with penicillinase added to the agar. On plates exposed in Room 2032, a reduction in the number of zones was observed in plates containing penicillinase. Of 10 plates without penicillinase exposed in Room 2032, four had so much antibiotic fallout that no bacterial growth occurred, and the other six had a total of 38 zones. In the 10 plates with the enzyme added, one plate had too many zones to count, but a total of only 18 zones was found in the other nine plates.

Zones were found only infrequently in plates exposed in other areas of the building, even in those where antibiotics were also handled (first and third floors). The only exception to this was Room B433A, a cleanup area servicing the entire building. Soap powders, antiseptics, and antibiotic-contaminated glassware were continuously processed there. Both test organisms are, to some degree, susceptible to antiseptics and most antibiotics.

The sensitivities to the test antibiotics were uniform throughout the testing periods. *S. lutea* sensitivity to penicillin was 0.001 unit, and *B. cereus* sensitivity to CTC was 0.0004 mcg.

In Phase II, recovery experiments indicated that when 0.06 unit of penicillin or 0.024 mcg. of CTC activity was placed on a membrane, 99% could be recovered. Table II shows that all areas in which some antibiotic fallout had been detected were negative when tested by air filtration, with the exception of Room B433A. Further testing was performed near this room. Air was sampled for 2.25 hr. with 72 cu. ft. of air passing through the membrane filter. The solution obtained after extraction of this filter was divided into two portions, and penicillinase was added to one portion. The solution was negative for the tetracyclines but positive for penicillin, as demonstrated by inactivation with penicillinase. The total activity on the filter was equivalent to 0.08 unit of penicillin G.

As shown in Table III, some air contamination was detected as fallout in areas where antibiotics were actively handled in operations such as weighing of antibiotic powders and blending of solutions. Table IV lists the results of filtration sampling of the areas indicated by fallout plates to have antibiotics in the air. The highest concentrations detected were equivalent to 0.012 unit of penicillin and 0.0185 mcg. of CTC per cu. ft.

In Phases I and III, a total of 378 plates was exposed on the second floor. Of these, 153 were positive and showed 426 countable particles, with 25 plates containing too many spots to count. Of 504 plates exposed in other areas, 28 were positive with a total of 56 particles detected. Of these, half were detected in the area of Room B433A.

DISCUSSION

Over 260,000 analytical determinations were made by NCAIA during the course of testing more than 20,000 batches of antibiotics over a 12-month period. These tests involved weighing, blending, and other handling of antibiotic powders and solutions that tend to release particles and aerosols. This study was undertaken to determine whether the handling and testing of antibiotic drugs constitute a source of contamination to the environment of the building.

The air-filtration system selected for this study is more stringent than that specified for monitoring airborne particulate contamination levels in open atmospheres, clean rooms, and other manufacturing and assembly areas, as described in the requirements of American Society for Testing and Materials Specification F25-63T and Society of Automobile Engineers Specification ARP743 for microscopic particle counting as referenced in the *USAF Technical Order 00-25-203 Revised;* a 0.8-μ porosity filter is satisfactory for these requirements. The 0.22-μ porosity filter used in this study is superior to that requirement.

Only small amounts of antimicrobial activity were found outside NCAIA except in the area of Room B433A. However, when these areas were subjected to quantitative assay of the air, the results

showed that, outside of NCAIA, antibiotics were detected only in the neighborhood of Room B433A. This basement location is in the hallway between the cage-washing operation and the glassware-washing area of the Laboratory Services Branch.

The antibiotic activity found in the air on the second floor, the largest area occupied by NCAIA, frequently appeared to be due to antibiotics other than penicillin. This conclusion is based on a comparison of results from assay plates with and without penicillinase. Although two spots of penicillin were found on one plate of four exposed in Room 1016 where penicillin contamination testing was performed, the many actual and control tests performed in that room have given no indication that environmental penicillin is ever detected by these tests. Most of the air coming into this room is filtered through Cambridge absolute filters to remove particles down to 0.3 μ in diameter.

This study shows that even in rooms occupied by NCAIA, there is no widespread heavy contamination of antibiotics in the air. Significant amounts occurred only in rooms where antibiotic powders were opened for weighing or where the drugs were extracted from dosage forms by blending. However, the quantities were not great in relation to the volume of air. The highest concentration of penicillin was 0.012 unit/cu. ft. and that of CTC was 0.0185 mcg./cu. ft. These small amounts would not affect the outcome of assays being performed as part of the certification program.

CONCLUSIONS

The incidence of contamination by antibiotic dust is negligible outside of the actual laboratory rooms where testing is performed. Therefore, persons in this building not testing antibiotics are not exposed to them by way of the atmosphere. Analysts testing antibiotics are exposed to a minimal degree.

REFERENCES

(1) U. S. Public Health Service, "Sampling Microbiological Aerosols," Communicable Disease Center, Technical Development Laboratories, and the Physical Defense Division, U. S. Army Chemical Corps; Public Health Service Publication No. 686, April 1959, pp. 1–53.

(2) G. Shaffer and J. McDade, *J. Amer. Hosp. Ass.*, **38**, 16(1964).

(3) Federal Standard No. 209, Clean Room and Work Station Requirements, Controlled Environment, December 16, 1963.

(4) National Aeronautics and Space Administration, "NASA Standards for Clean Rooms and Work Stations for the Microbially Controlled Environment," NHB 5340.2, August 1967.

(5) H. Welch, W. W. Wright, R. J. Reedy, E. J. Oswald, and D. M. Wintermere, *Antibiot. Annu.*, **1958–1959**, 942.

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Conformation of Some Acetylcholine Analogs as Solutes in Deuterium Oxide and Other Solvents

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Abstract □ The proton magnetic resonance spectra of α- and β-methylacetylcholines in deuterium oxide are described and analyzed. Chemical shift and coupling constant data derived from these analyses and from the spectra of model compounds establish that β-methylacetylcholine shows a preference for *gauche* $^{+}$N/O conformers, while the α-isomer displays a lack of conformational preference in the solute condition. Additional IR and proton magnetic resonance studies of acetylcholine and some analogs of acetylcholine, in which one *N*-methyl is replaced by a bulkier group, uphold the preferred nature of *gauche* $^{+}$N/O conformers in the parent compound as a solute and support an intramolecular $^{+}$N. . . OCOMe interaction as a factor governing conformational preferences in these esters. The significance of the results in terms of the influence of conformational isomerism upon the duality of action of acetylcholine is discussed.

Keyphrases □ Acetylcholine analogs—conformation determination in deuterium oxide □ α-, β-Methylcholine—conformational analysis □ Conformation—acetylcholine analogs □ PMR spectroscopy—structure □ IR spectrophotometry—analysis

Knowledge of the conformation of acetylcholine (Ach) and related agonists has been increasingly sought during the last few years. Such information, while of potential value to the delineation of receptor characteristics and agonist–receptor interactions (1), is of additional importance in the cholinergic field because of recent postulates associating muscarinic and nicotinic effects of Ach with different conformations of the Ach molecule, recently reviewed (2). Reports upon the conformation of Ach and its congeners chiefly relate to the solid sate. Canepa *et al.* (3) showed that Ach bromide has a *gauche* $^{+}$NCCO conformation; this arrangement also occurs in many related compounds such as α-glycerophosphorylcholine and muscarine iodide (4), all in the solid state. Solute conformations of Ach in ethanol (5) and heavy water (6) have been advanced on the basis of infrared (IR) and proton magnetic resonance (PMR) spectroscopic data, respectively, the latter study in particular providing good evidence for a preferred *gauche* conformation. Molecular orbital calculations for Ach lead to the same prediction of preferred conformation (7).

This article reports some spectroscopic studies relating to the solute conformations of α- and β-methylacetylcholines analogs which retain, respectively, the nicotinic and muscarinic properties of Ach (8); the solid-state conformations of both compounds recently were described (9). Some studies of Ach analogs in which *N*-methyl is replaced by a bulkier substituent are also described.

PMR SPECTROSCOPY

PMR evidence of the conformation of substituted ethanes requires knowledge of the magnitude of spin-spin coupling between